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Cytotoxicity Screening and Invitro Antioxidant Potential of *NEWBOULDIA LAEVIS* Leaf Extract

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Abstract
This study was aimed at determination of the phenolic composition of phenolic, antioxidant activity and cytotoxicity *Newbouldia laevis* leaf extract to brine shrimps (*Artemia salina*). Extracts from the leaves of the plant was subjected to phytochemical screening to determine the total phenolic contents. The methanolic extract had the highest percentage yield after extraction and phenolic content phenolic content of 22.844mg/ml, followed by chloroform, petroleum ether and ethyl acetate extracts with 0.200, 0.601, 1.803mg/ml respectively, hence, the methanolic extract was used for further investigation. The effect of the methanolic extract in scavenging free radical was measured using 2, 2-diphenyl-1-piecryl hydrazyl (DPPH) radical was determined spectrophotometrically. Results revealed that the methanolic extract had IC_{50}=176.28µg/ml while the standard vitamin C had IC_{50}=76.9µg/ml. The result of brine shrimp lethality assay of the extract were relatively safe (LC_{50}=2690.3µg/ml) when compared with the reference potassium dichromate (LC_{50}=629.93 µg/ml). This report indicates that the leaf of the plant is relatively safe for the purposes utilized in this study, hence are potential antioxidant source that could offer protection against oxidative stress.

Keywords: *Newbouldia laevis*, leaf extract, *Artemia salina*, methanolic extract, free radical, Vitamin C, Potassium dichromate, brine shrimp lethality assay.

INTRODUCTION:

Plants are put to medicinal use all over the world since time immemorial. The importance of medicinal plants and traditional health systems in solving healthcare problems of the world is gaining increased attention [1]. They are among the most important and common sources of food and potentially new drugs [2]. The medicinal value of plants lies on some chemical substances that produce a definite physiological action on the human body which include phenolic compounds, alkaloids, tannins and flavonoids [3, 4]. Phenolic compounds of plants generally, have been reported to play key antioxidant roles, especially using the mechanism of delocalization of the single electron of the radical [5]. Antioxidant substances block the action of free radicals which are implicated in the pathogenesis of many diseases [6]. DPPH has been widely used to evaluate the antioxidant activity of natural products from plant and microbial sources [7].

Newbouldia laevis is commonly known as African border tree. In Nigerian major languages it is called 'Aduruku' in Hausa, 'Ogirisi' in Igbo and 'Akoko' in Yoruba [8]. *N. laevis* is a medium sized, sun loving, fast growing drought tolerant angiosperm which belongs to the Bignoniaceae family [9]. *Newbouldia laevis* is used in various ethnomedicinal preparations across Africa. The root bark is used in the treatment of enlarged spleen, dysentery, worm infestations, migraine, ear ache, conjunctivitiy, and various forms of orchitis [10]. Numerous research have been carried out on various parts of *Newbouldia laevis* to know more about its uses for therapeutic purposes against a number of diseases [10, 11]. Leaf and root extracts of *N. laevis* have been shown to possess antimalarial [12, 13] and antimicrobial activities [14, 15]. Sedative effects of methanolic leaf extract of *N. laevis* in mice and rats have also been studied and reported [16]. In other to study the toxicity of this medicinal plant, lethality bioassay which is based on the ability

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to kill laboratory cultured shrimps (*Artemia salina*) is performed. The brine shrimps assay is a very useful tool for the isolation of bioactive compounds from plant extracts [17].

MATERIALS AND METHODS

Newbouldia laevis (Bignoniaceae) leaves were collected directly from the trees in Kogi state University environment. They were identified and authenticated at the botany unit in Biological science department, Kogi state University, Nigeria. The leaves were air-dried and ground into fine powder and kept in non-absorptive nylon for subsequent use.

Preparation of plant extracts

Methanolic, chloroform, ethyl acetate and petroleum ether extract were prepared by weighing and mixing the preparation in the ratio 1:10 of sample to each solvent respectively. It was left for 72 hrs and shaken at intervals. The crude extract was then decanted, filtered and concentrated using rotary evaporator until the solvent was completely removed. Weight of concentrated dry extract was recorded for yield and percentage yield calculations. The solid residue was stored in glass vials in a refrigerator.

Quantification of total phenolic compound

The total phenol composition of the extracts was determined using Folin-Dennis reagent as described by the modification of Gow Chin Yen and Pin – Der Dur method (1994) [18]. 0.3g of each of the four extracts were weighed into four test tubes each. 0.45g of the standard was also weighed into a fifth test tube. 50% ethanol was then added to all 5 tubes. It was then put on whirl mixer for some minutes. Chloroform and ethyl acetate extract was put in water bath briefly to aid the dissolution. All test tubes were kept for 3hrs and shaken on the whirl mixer at intervals. The filtrates were then collected into 100ml conical flasks. 20µl of filtrate was pipetted using a micropipette into a 50ml flask and 2.5ml of Folin Dennis reagent was added and the blank was also prepared. All flasks were made up to the mark with distilled water and left for 30 minutes. The absorbance was then read with spectrophotometer at 517nm against a DPPH control prepared and the total phenol content was then calculated.

Antioxidant activity of plant extract (DPPH radical scavenging activity)

Antioxidant activity of the plant extract was carried out using the free radical scavenging assay by DPPH (2, 2-diphenyl-1-picrylhydrazyl) with the modified method described by Blois (1985) [19]. 0.3mM DPPH was first prepared 0.01g of the methanolic extract was weighed and dispensed in 10ml of methanol. Same preparation was also made for the standard (vitamin C). 1ml each of both standard and sample extract was then pipetted into two test tubes and 1ml methanol was then added. Serial dilution was then made with varied concentrations of 1000, 500, 250, 125, 62.5ul/ml for both the extract and standard. The reaction mixture was incubated for 30min at room temperature; the absorbance was recorded at 517 nm against a DPPH control prepared. The annihilation activity of free radicals was calculated using:

% of Inhibition= (Absorbance of control – Absorbance of Test)/Absorbance of control * 100

The IC_{50} was calculated using the linear regression analysis.

Cytotoxicity bioassay

The cytotoxicity bioassay of the methanolic extract of Newbouldia laevis was carried out using the modified method of Solis *et al.*, (1992) [17] and Potduang *et al.*, (2007) [20]. Brine shrimps (*A. Salina*) were hatched using brine shrimp eggs in a plastic vessel (500ml) filled with sterile artificial sea water prepared using sodium chloride (16g/l) and adjusted to pH 8.5 under artificial aeration for 24hrs with a constant light source supplied. After hatching, active nauplii free from egg shells were drawn through micropipette and placed in vials containing 10ml of sterile artificial sea water. 1ml of the stock solution of the extract was then used to make serial dilution of varied concentrations of 1000, 500, 250, 125, 62.5 µg/ml. The standard potassium dichromate was also prepared (0.01g of the standard with

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10ml of methanol) and the concentration was also varied as that of the extract.
50µl each of the two varied concentrations were then added to the hatched brine shrimps in the vials 10 each for all the two varied concentrations of the standard and extract. It was then incubated for 24hrs at room temperature. The living shrimps were then counted under a hand magnifying lens. Percentage lethality was calculated and LC_{50} was calculated using the regression analysis.

RESULTS AND DISCUSSION

Significant increase was observed in the yield and percentage yield of the methanolic extract of *Newbouldia laevis* presented in Table 1 with values 5.02g and 10.04% respectively when compared with the chloroform, petroleum ether and ethyl acetate extracts. Same was observed for the quantitative estimation of the phenolic composition of the crude extract of *N. laevis* methanolic leaf extract having the highest concentration (22.844mg/g) compared to that present in the chloroform, petroleum ether and ethyl acetate extracts with values 0.20mg/g, 0.601mg/g and 1.803mg/g respectively as shown in Table 2. Phenolic moieties present in the molecular structure of natural antioxidants often help in enhancing their activity [21]. Phenols possess hydroxyl groups which are responsible for free radical scavenging effects. Thus, they act as free radical chain terminators; thereby acting as antioxidants [5].

Table 1. Percentage yield of leaf extracts

Extraction	Yield (g)	Percentage yield
Methanol extraction (50g/500ml)	5.02	10.04
Chloroform extraction (30g/300ml)	2.54	8.46
Ethyl acetate extraction (50g/500ml)	3.05	6.1
Petroleum ether extraction (40g/400ml)	3.32	8.3

Table 2: Total phenolic contents of leaf extracts

Sample	Absorbance 760nm	Phenolic concentration (mg/g)
Methanolic extract	0.114	22.844
Chloroform extract	0.001	0.200
Ethanol extract	0.0009	1.803
Petroleum ether extract	0.0003	0.601

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Table 3: Result of antioxidant activity of methanolic leaf extract of *N. laevis* leaves and vitamin C

Test sample	Concentration (µg/ml)	Log concentration	Percent Inhibition (%)	IC_{50}
Methanolic extract	1000	3.000	88	IC_{50}=176.28 µg/ml
	500	2.6990	67	
	250	2.3979	55	
	125	2.0969	52	
	62.5	1.7959	24	
Vitamin C (ref)	1000	3.000	80	IC_{50}=76.9 µg/ml
	500	2.6990	61	
	250	2.3979	48	
	125	2.0969	34	
	62.5	1.7959	27	

Table 4: Results of brine shrimps lethality assay on methanol leaf extract and control

Test sample	Concentration (µg/ml)	Number of Death Recorded	Percentage Lethality	LC_{50}
Methanol extract	1000	4	40	LC_{50} = 2690.31 µg/ml
	500	3	30	
	250	3	30	
	125	2	20	
	62.5	1	10	
Potassium dichromate (Positive control)	1000	6	60	LC_{50} = 629.93 µg/ml
	500	4	40	
	250	4	40	
	125	3	30	
	62.5	2	20	

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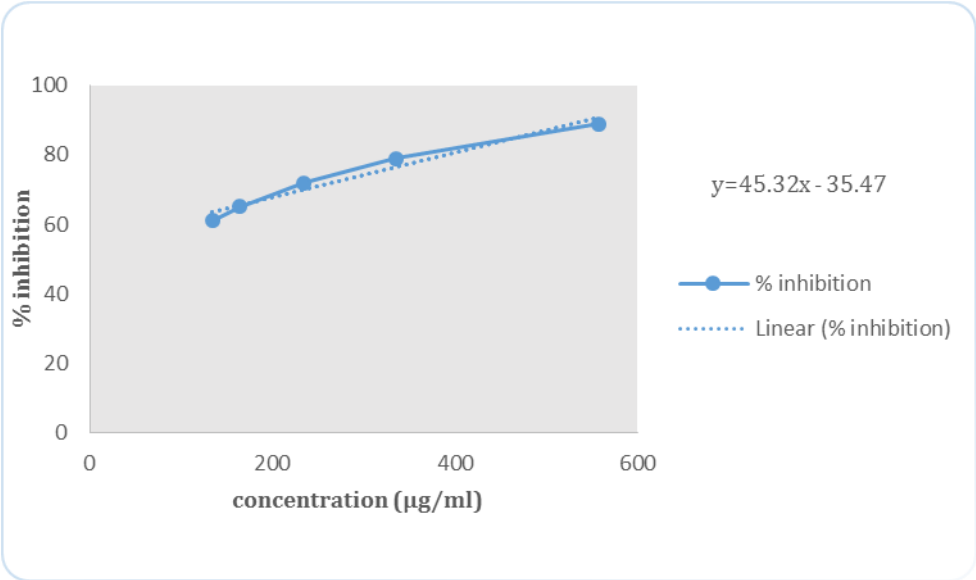

Fig.1: Graphical representation of concentration (µg/ml) vs percentage inhibition of methanolic solution of Vitamin C (standard antioxidant)

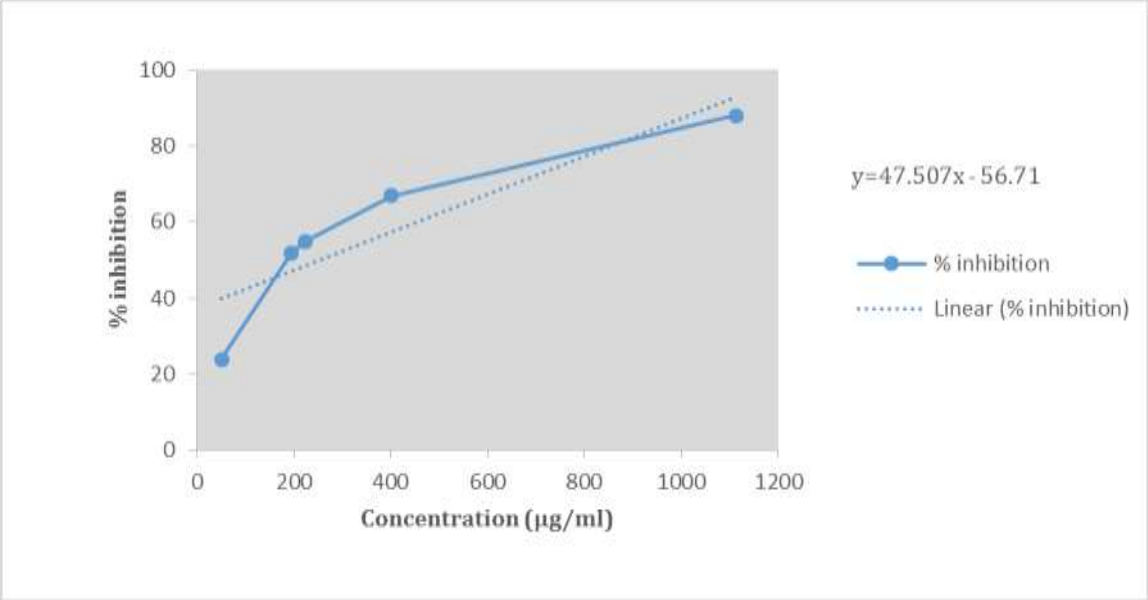

Fig.2: Graphical representation of concentration (µg/ml) vs percentage inhibition of methanolic solution of crude extract

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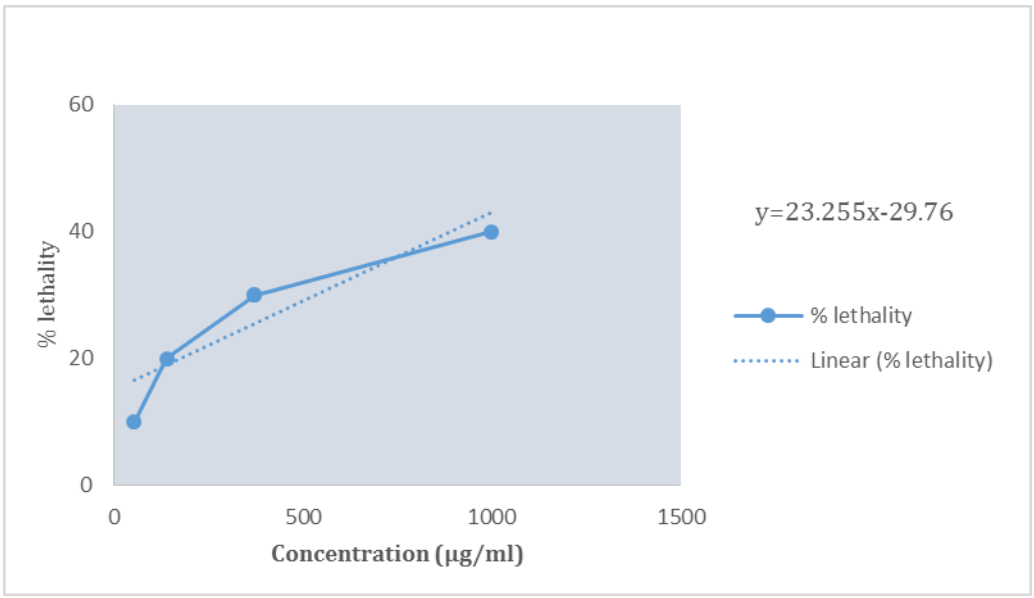

Fig.3: Graphical representation of concentration (µg/ml) vs percentage lethality of methanolic solution of crude extract

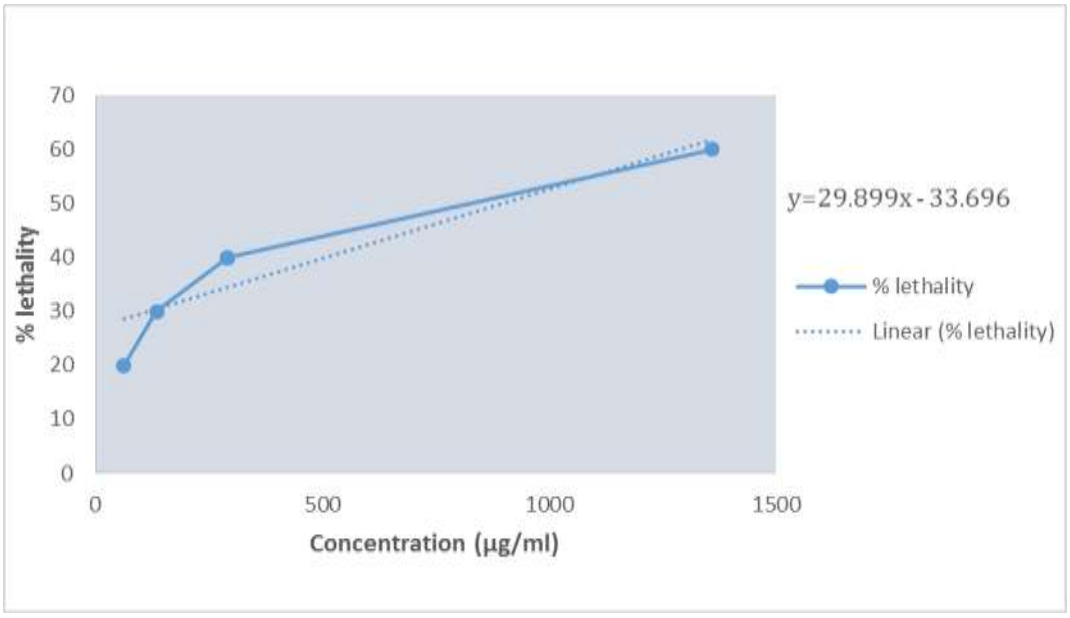

Fig.4: Graphical representation of concentration (µg/ml) vs percentage lethality of methanolic solution of Potassium dichromate (standard)

The values presented in Table 3 shows the antioxidant activities of *N. laevis* which was determined using the free radical scavenging activity by DPPH (2,2-Diphenyl-1-picrylhydrazyl) compared with the activity of ascorbic acid, a known antioxidant. DPPH is known to be a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [22]. Scavenging of DPPH radical is the basis of the popular

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DPPH antioxidant assays [23]. Methanolic leaf extract at various concentration (1000, 500, 250, 125 and 62.5µg/ml), showed antioxidant activities in a concentration dependent manner. It was observed that the extract and reference antioxidant exhibited strong activity as concentration increased. The methanol extract at the concentration of 1000µg/ml for *N. laevis* showed an antioxidant activity (88%) higher than that of 1000 µg/ml of ascorbic acid (80%), the reference compound. The DPPH assay method is used to evaluate antioxidant potential of plant extracts, foods and pure compounds [24]. The results of the Brine Shrimp lethality assay using the methanol extracts from the Leaves of *N. laevis*, the percentage lethality and the LC_{50} values obtained for extracts and that of the positive control, Potassium dichromate are given in Table 4. The methanol extract of *N. laevis* showed mild brine-shrimp inhibition when compared with the control (Potassium dichromate) used. The LC_{50} of the extract was 2690.31µg/ml compared to 629.93 µg/ml of the control. Maximum mortality took place at a concentration of 1000 µg/ml for both extracts as well as for the positive control. The present finding is in agreement with the investigation carried out by Daniel and Ekam (2016) [25] indicating that Brine Shrimp lethality of plant extracts were found to be concentration-dependent. Different pharmacological properties can be assumed on the basis of Brine Shrimp toxicity of any plant extract [34].

CONCLUSION

On the basis of the results obtained from this study, it is evidenced that methanol extract of *Newbouldia laevis* has significant amount of phenol which is a potential source of antioxidants and may be favourably used for the treatment of diseases caused by reactive oxygen species because antioxidants serves as radical scavengers.

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